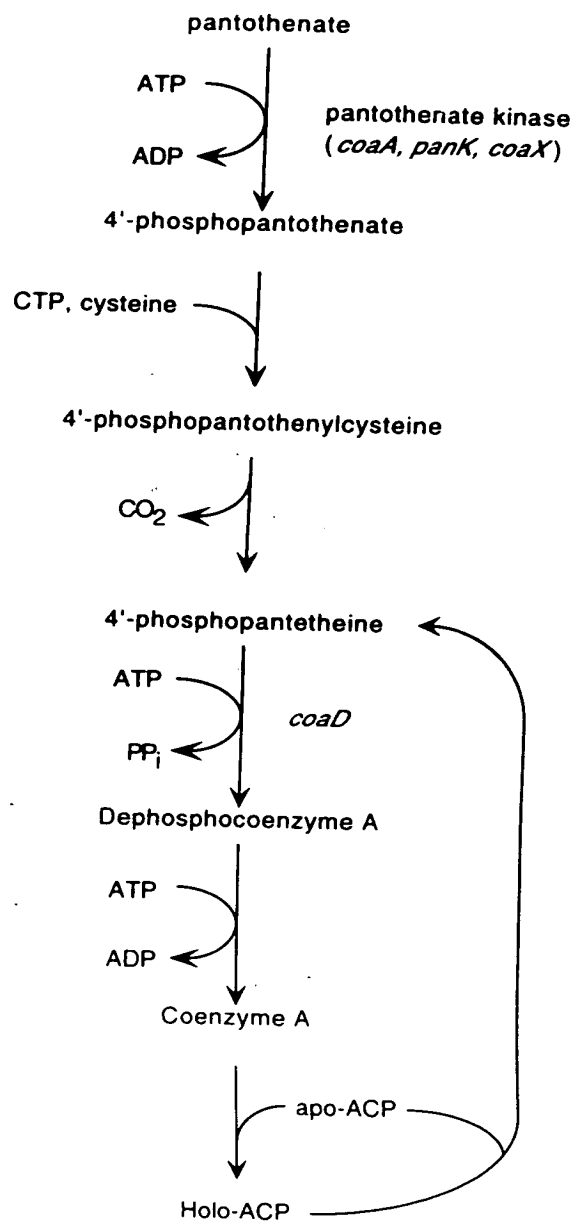
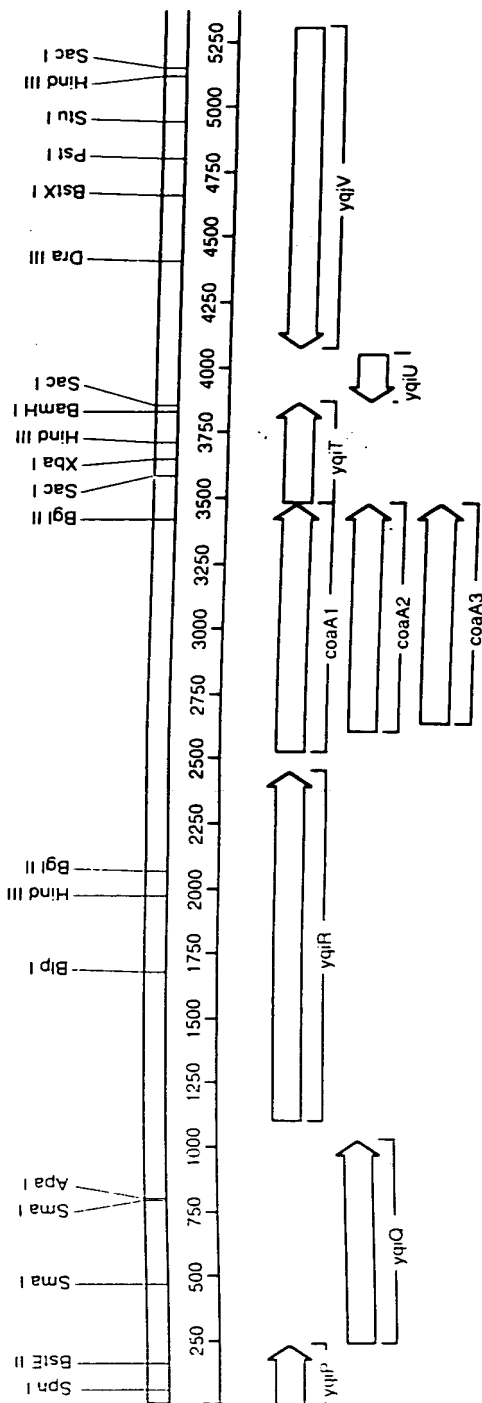


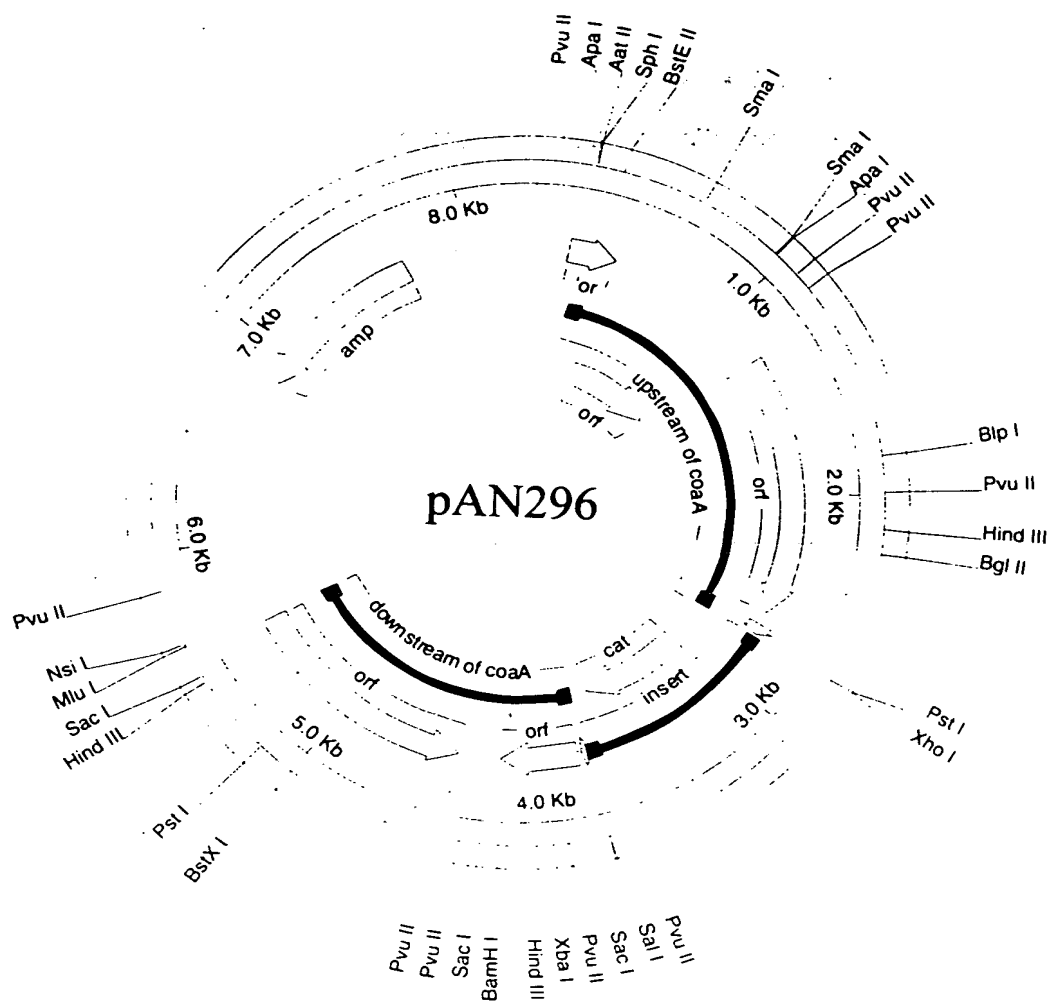
[illegible]

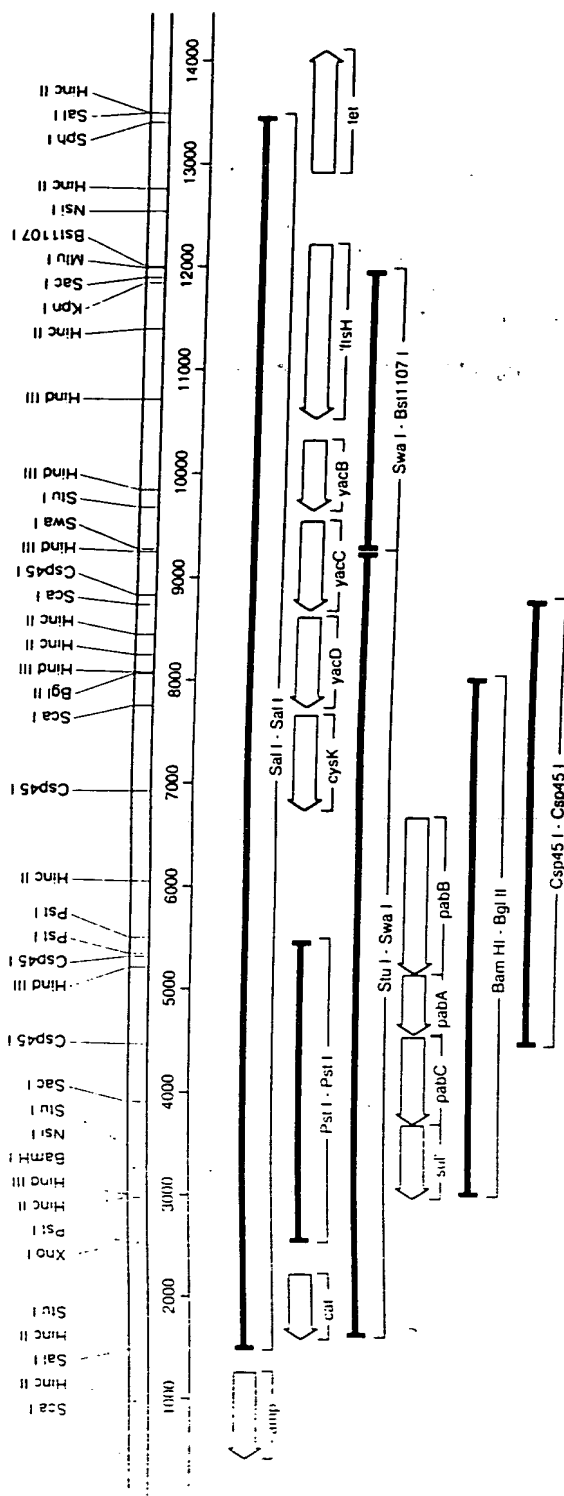
2

Figure 1 Structure of the *B. subtilis* chromosome in the region of the *coaA* gene. The scale is in base pairs and the significant open reading frames are shown by the open arrows.

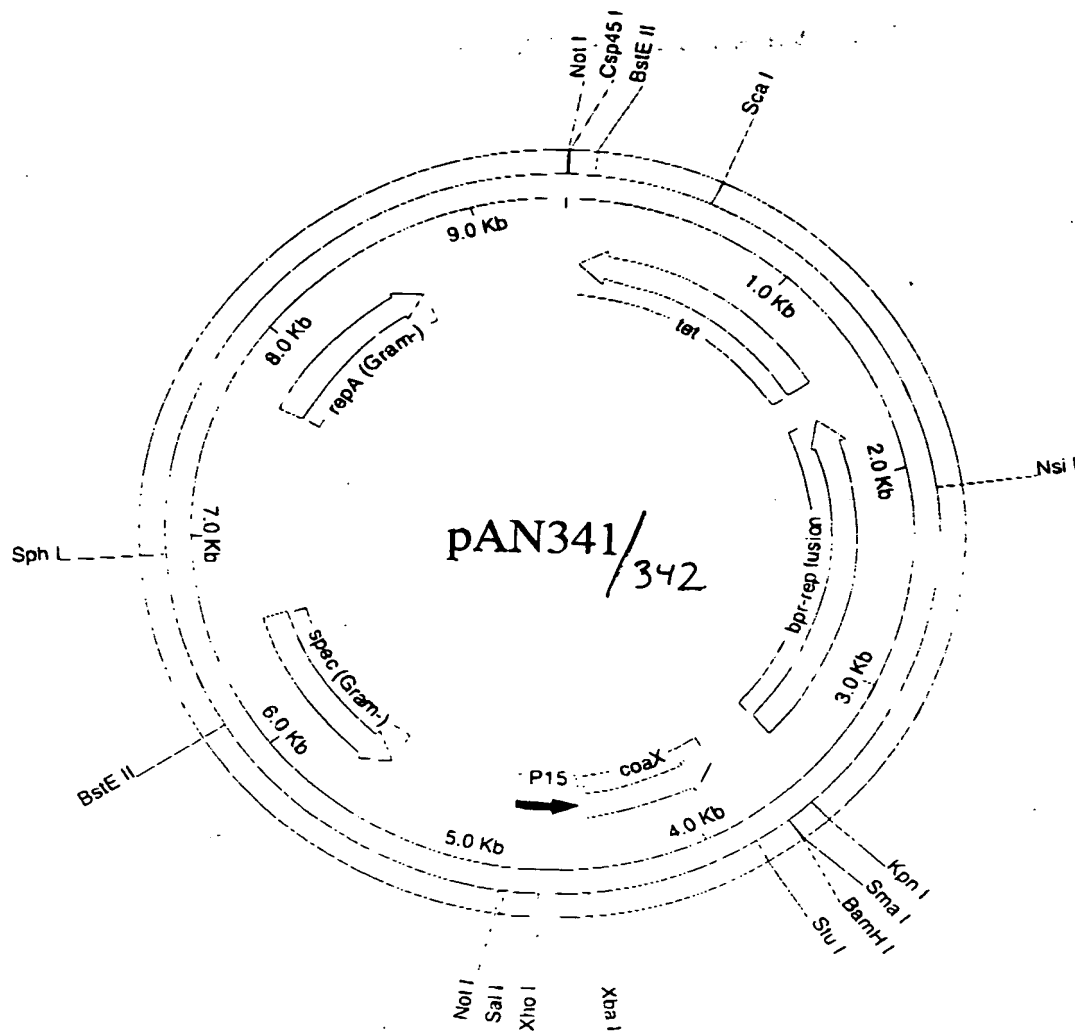


15
 Figure 17 Structure of pAN296, designed to delete most of the *B. subtilis* *coaA* gene and substitute a chloramphenicol resistance gene.





5
 Figure 27 Structure of pAN341 and pAN342, two independent PCR-derived clones of *yacB* (renamed *coaX*).



6A

FIG. 28A

CLUSTAL W (1.7) Multiple Sequence Alignments

Sequence type explicitly set to Protein

Sequence format is Pearson

Seq. 1: B.subtilis|Coax|SEQNO. 258 aa Seq. 8: sp|O51477|B.burgdorferi 262 aa
Seq. 2: dbj|BAA21476.1|D.vulgaris 212 aa Seq. 9: sp|P74045|Synechocystis 257 aa
Seq. 3: gb|AAD35964.1|T.maritima 246 aa Seq. 10: sp|O25533|H.pylori 223 aa
Seq. 4: pir|T36391|S.coelicolor 265 aa Seq. 11: sp|O67753|A.aeolicus 229 aa
Seq. 5: sp|Q45338|B.pertussis 267 aa Seq. 12: sp|Q9RX54|D.radiodurans 262 aa
Seq. 6: sp|O06282|M.tuberculosis 272 aa Seq. 13: WIT|RCA03301|C.acetobutylicum 250 aa
Seq. 7: sp|O83446|T.pallidum 273 aa Seq. 14: WIT|RRC02473|R.capsulatus 258 aa

B.subtilis|Coax|SEQIDNO. 2
WIT|RCA03301|C.acetobutylicum
pir|T36391|S.coelicolor
sp|O06282|M.tuberculosis
WIT|RRC02473|R.capsulatus
dbj|BAA21476.1|D.vulgaris
sp|Q9RX54|D.radiodurans
gb|AAD35964.1|T.maritima
sp|O83446|T.pallidum
sp|O51477|B.burgdorferi
sp|O67753|A.aeolicus
sp|P74045|Synechocystis
sp|O25533|H.pylori
sp|Q45338|B.pertussis
-----MLLVIDVGNNTVTLGVYHDG-----KLEYHWRIE
NKRAAFMLLLFLRSVLKVLVLDVGNNTNIVLGIYNDT-----KLTAEWRLS
-----MLLTIDVGNTHTVLGLFDGE-----DIVEHWRIS
-----MLLAIDVRNTHTVVGLSGMKEHAKVWQWMRIR
-----MLLICDCGNTNTVFSVMDGT-----DFAATWRIA
-----MTQHFLFDIGNTNVKGIAVET-----AVLTSYVLP
-----MPAPPLLAVIDIGNTTTVLGLADAG-----ALHTWIR
-----MYLLVDVGNTHSVFSITEDG-----KTFRRWRLS
-----MLLIDVGNSHVVFGIQGNGRVCVRELFRLA
-----MKNPLSELIDIGNTSIAFALKDN-----QVNLFIKMK
-----MRELTVDVGNSSVDIALWEGK-----KVK
-----METSKEGCGALDNDKQKPLGLMIGN-----SRLHWAYC
-----MPARQSTDLKN-----LVLCIDIGN-----TR
-----MIILIDSGNSRLKVGWFDPDAP--QAAREPAPV

6B

FIG.23B

B. subtilis|Coax|SEQIDNO_2
WIT|RCA03301|C.acetobutylicum
pir|T36391|S.coelicolor
sp|O06282|M.tuberculosis
WIT|RR02473|R.capsulatus
dbj|BAA21476.1|D.vulgaris
sp|Q9RX54|D.radiodurans
gb|AAD35964.1|T.maritima
sp|O83446|T.pallidum
sp|O51477|B.burgdorferi
sp|O67753|A.aeolicus
sp|P74045|Synechocystis
sp|O25533|H.pylori
sp|Q45338|B.pertussis

B. subtilis|Coax|SEQIDNO_2
WIT|RCA03301|C.acetobutylicum
pir|T36391|S.coelicolor
sp|O06282|M.tuberculosis
WIT|RR02473|R.capsulatus
dbj|BAA21476.1|D.vulgaris
sp|Q9RX54|D.radiodurans
gb|AAD35964.1|T.maritima
sp|O83446|T.pallidum
sp|O51477|B.burgdorferi
sp|O67753|A.aeolicus
sp|P74045|Synechocystis
sp|O25533|H.pylori
sp|Q45338|B.pertussis

TSRHKTEDEFQMILRSFDHS-----GLMFEQIDGIISSVVPPIMFALER
TDVLSADEYGIQVNNLFQD-----KLDPTLVEGVISSVVPMIYSLEH
TDSRRTADELAVLLQGLMGMHPLGLDELGDIGDIAICATVPSVLHELRE
TESEVTADELALTIDGLIG-----EDSERLTGTAALSTVPSVLHEVRI
TDHRRTADEYFWLNTLMQLK-----GLOGRISEAIISSAPRVVFNLRV
TDPGQTDSIGRLLEVLRHAG-----LGPADVACVASSVPPGVNPLIRR
TNREMLPDDIALALQHLGFLA-----CAP-IPRAAVLSSVAPPVGENVAL
TGVFQTEDELFSHLHPLGL-----DAMREIKGIGVASWVPTQNTVIER
PDARKTODEYSLLIHALCERAG-----VGRASLRDAFISSVPLTKTIAD
TNMLRLYDEYVSFFEEFNDFN-----VN---K-VFISSVWPILNETFKN
DFLKLSHHEFLKEEPPKLK-----ALGISVKQSFSEKVRG
SGNAPLQTVWTDYNPKSAQLP-----VLLGKVPLMLASVVPE
IHFAQNYQLFSSAKEDLKR-----LGIQKEIFYISVNEE
AFDNLDLDALGRWLATLPRRP-----Q-----RALGVNVVAGLARGEAIA

MCTKYFHIERQIVG-PG-MKTGLNINIKYDNPKEVGADRIVNAVAAIHLYG-
MIRKYFKINPLWVG-PG-IKTGINIKYDNPKEVGADRIVNAVAAHEIYK-
VTRRYGDDVPAVLVEPG-VKTGVPILTDHPKEVGADRIINAVAAVELYG-
MLDQYWPSPVHLTEPG-VRTGIPLLVDNPKEVGADRIVNCALAYDRFR-
LCNRYFDCRPYVVGKPG-CELPVAPRVDPGTTGVPDRLVNTVAGYDRHG-
ACERYL--YRKLLEFAPGDIAIPLDNRYERPAEVGADRLVAAVAAARLYP-
ALKRHFMDAFVSAEN--LPDVTVELDTGCSVGADRLCNLFCAEKYLG-
FSQKYPHISPIWVKA--GCVMNVKNPSEVGADRVANVAVAFVKEVG-
AVAQISGVQPVFGPWAYEHLVRIPEPVRAEIGTDLVANAVAAVYVHFR-
VIFSFKIKPLFIGFDLNYDLTFNPKSKDFLLGSDVFNANLVAAIENYS-
KIPKIK-----FLKKEN--PFIQVDYKTPETLGTDRVALAYSAKKPYG-
QTEVWRVYQPKILTKN--LPLVNLYP---SFGIDRALAGLGTGLTYG-
NEKALLNCVFNAKNIAG--FFHLETDYVG---LGDIDRMACIA---VN--
ATLRAGGCDIRWLRAQP-LAMGLRNGYRNPDLGADRWACMGVGLARQPS

6c

FIG. 28C

2
B. subtilis|Coax|SEQIDNO. 2
WIT|RCA03301|C. acetobutylicum
pir|T36391|S. coelicolor
sp|O06282|M. tuberculosis
WIT|RC02473|R. capsulatus
dbj|BAA21476.1|D. vulgaris
sp|Q9RX54|D. radiodurans
gb|AAD35964.1|T. maritima
sp|O83446|T. pallidum
sp|O51477|B. burgdorferi
sp|O67753|A. aeolicus
sp|P74045|Synechocystis
sp|O25533|H. pylori
sp|Q45338|B. pertussis

2
B. subtilis|Coax|SEQIDNO. 2
WIT|RCA03301|C. acetobutylicum
pir|T36391|S. coelicolor
sp|O06282|M. tuberculosis
WIT|RC02473|R. capsulatus
dbj|BAA21476.1|D. vulgaris
sp|Q9RX54|D. radiodurans
gb|AAD35964.1|T. maritima
sp|O83446|T. pallidum
sp|O51477|B. burgdorferi
sp|O67753|A. aeolicus
sp|P74045|Synechocystis
sp|O25533|H. pylori
sp|Q45338|B. pertussis

NP--LIVVDFGTATTTCYIDENKQYMGGAIAPIGTTISTEALYSRAAKLPR
RS--LIIDFGTATTTCAVRENGDYLGGAIICPGIKVSEALFEKAALPR
GP--AIVVDFGTATTTDAVSARGEYIGGVIAPGIEISVEALGVKGAQLRK
KA--AIVVDFGSSICVDVVSAKGEFLGGAIAPGVQVSSDAAAARSAAALRR
GD--LIVVDFGTATTTFDVAPDGAIVGGVIAPGVNLSLEALHMAAALPH
GPRSLVDFGTATTTDCVEG-GAYLGGILCPGLVSSAGALSSRTAKLPR
GLDYAVVDFGTSTNFDVVGRRFLGGILATCAQVSADALFARAALPR
KN--GIIDMGATTTVDLVN-GSYEGGAILPGFFMVHSLFRGTAKLPL
SA--CVVDDCGTALTAVDGTGLIQVAIAPGLRTAVQSLHTGTAQLPL
FEN-VLVVDLGTACTIFAVSRQDGLGGIINSGLPLNFNSLLDNAYLIKK
KN--VVVISAGTALVIDLVE-GKPKGPFITLGGKKLKILSDLAEGIPE
FP--CLVVDGGTALTITGPDQDKLVGGAILPGLGLQLATLGDRLAALPK
NG---VVVDAGSAITIDLIKE-GRHLGGCILPGLAQVIHAYKKSAKILEQ
VHPPLLVASFGTATTLDTIGPDNVFPGGILPGPAMMRGALAYGTAHLPL
: . * . :

IEITRPDN---IIGKNTVSAMQSGILFGYVQVEGIVKRMKQAKQDLK-
VELIKPAY---AICKNTISSIQSGIIVRYLRQVKYLFKELKENLPDGRRT
IEVARPRS---VIGKNTVEAMQSGIIVYGFAGQVDGVVNRMARLADD--P
VELARPRS---VVGKNTVECMQAGAVFGFAGLVGIRIREDVSGFSVD
VDVTKPGQ---VIGTNTVACIQSGVYWGVI GLVEGIVRQIRMERDRP---
ISLEVEDS-PVIGRSTTTSLNHGFI FGFAAMTEGVLA-----
ITLQAPET---AIGKNTVHALQSLGVFGYAEMVDGLLRIRRAELPGE---
VEVKPADP---VVGKDTTEENIRLGVWVGSVYALEGIIGRIKEVYGDLP---
VPLALPDS---VLGKDTTHAVQAGVVRGTLFVIRAMIAQCQKELGCR---
FPISTPNN---LLERTTSGVNSGLFYQVKYLIIEGVYVDIKQMYKKK---
FFPEEVEI---FLGRSTRECVLGGAYRESTEFIKSTLKLWRKVFKRK---
LEMDQLTELPRWALDTPSAIFSGVYGVGLGALQSYLDQWQKLFPGA---
PFKALDSL--EVLPKSTRDAVNVGMVLSVIACIQHLAK--NQK-----
ADGLVADY-----PIDTHQAIASGIAAQAAGAIVRQWLAGRQRYGQAP---

60

FIG. 23D

2
B. subtilis|Coax|SEQIDNO
WIT|RCA03301|C.acetobutylicum
pir|T36391|S.coelicolor
sp|O06282|M.tuberculosis
WIT|RRC02473|R.capsulatus
dbj|BAA21476.1|D.vulgaris
sp|Q9RX54|D.radiodurans
gb|AAD35964.1|T.maritima
sp|O83446|T.pallidum
sp|O51477|B.burgdorferi
sp|O67753|A.aeolicus
sp|P74045|Synecocystis
sp|O25533|H.pylori
sp|Q45338|B.pertussis

2
B. subtilis|Coax|SEQIDNO
WIT|RCA03301|C.acetobutylicum
pir|T36391|S.coelicolor
sp|O06282|M.tuberculosis
WIT|RRC02473|R.capsulatus
dbj|BAA21476.1|D.vulgaris
sp|Q9RX54|D.radiodurans
gb|AAD35964.1|T.maritima
sp|O83446|T.pallidum
sp|O51477|B.burgdorferi
sp|O67753|A.aeolicus
sp|P74045|Synecocystis
sp|O25533|H.pylori
sp|Q45338|B.pertussis

-----VIATGG-----LAPLIANES-----DCIDIVDPFLTKGLELI
RTSLVLTATGG-----LAKLIN-----
DDVTVIATGG-----LAPMVLGES-----SVIDEHEPWLTMGLRLV
HDVAIVATGH-----TAPLLPEL-----HTVDHYDQHLTLQGLRLV
--MKVIATGG-----LASLFDLGF-----DLFDKVEDDLTMHGLRLI

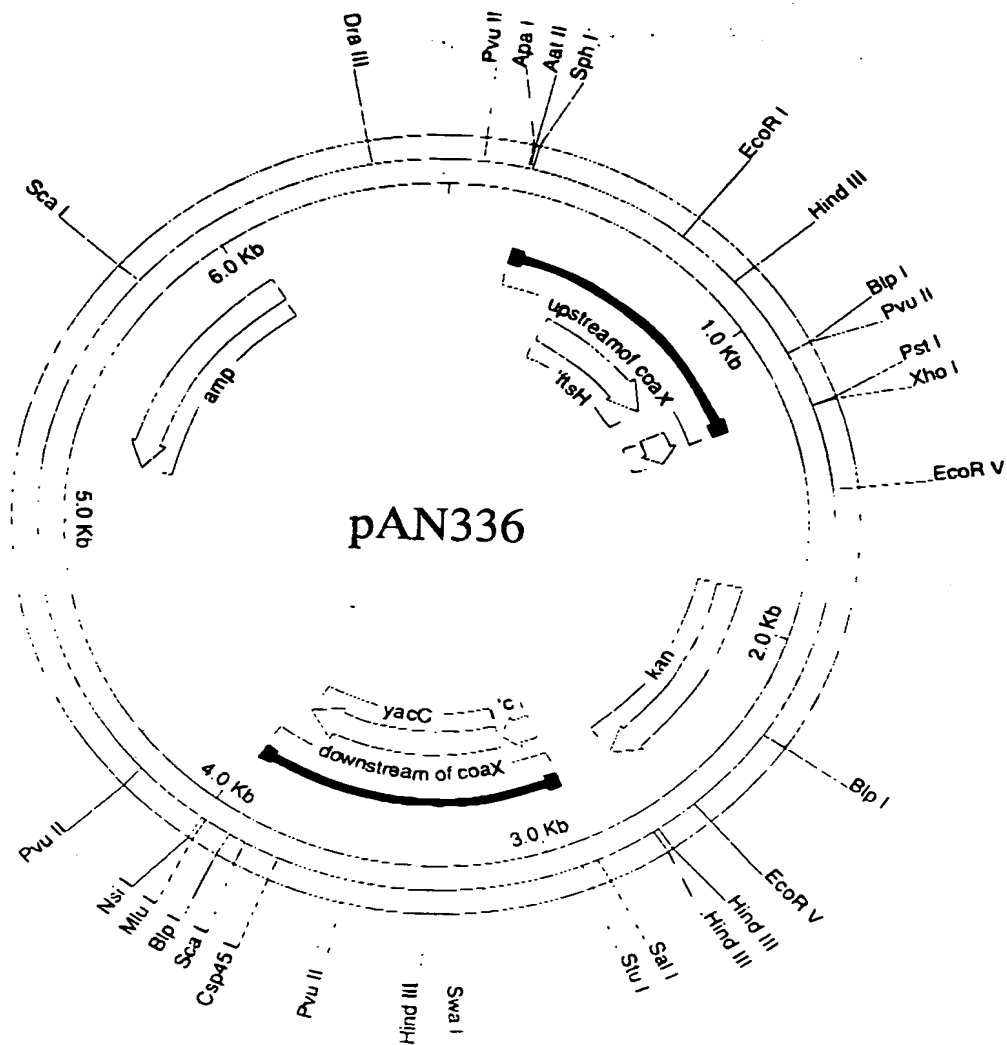
--AVAVATGG-----FSRTVQGC-----QEIDYDETLTLRGLVEL
-----VLTGG-----QSKIVK-DM-----IKHEIFDEDLTIKGVYHF
--CAAVITGG-----LSRLFS-SE-----VDFPPIDAQLTLSGLAHI
--FNLIITGG-----NADLISLI-----EIEFIFNIHLTVEGVRIL
--FKWITGG-----EGKYFS-----KFGIYDPLLVHRGMRL
--AMVITGG-----DGKILHGFLEKHSNLSVWDDNLIIFLGMAAI
---IYLCGG-----DAKYLSAFL-----PHSVCKERLVFDGMEIA
---EIVYAGGWPVEVRAERLLAVTGAAGATPQPTYLDSPLVDGLAAL

YERNRVGSV-----

YERNVSRM-----
FERNLEVOGRGLKTAR-----
FDYNGKLG-----

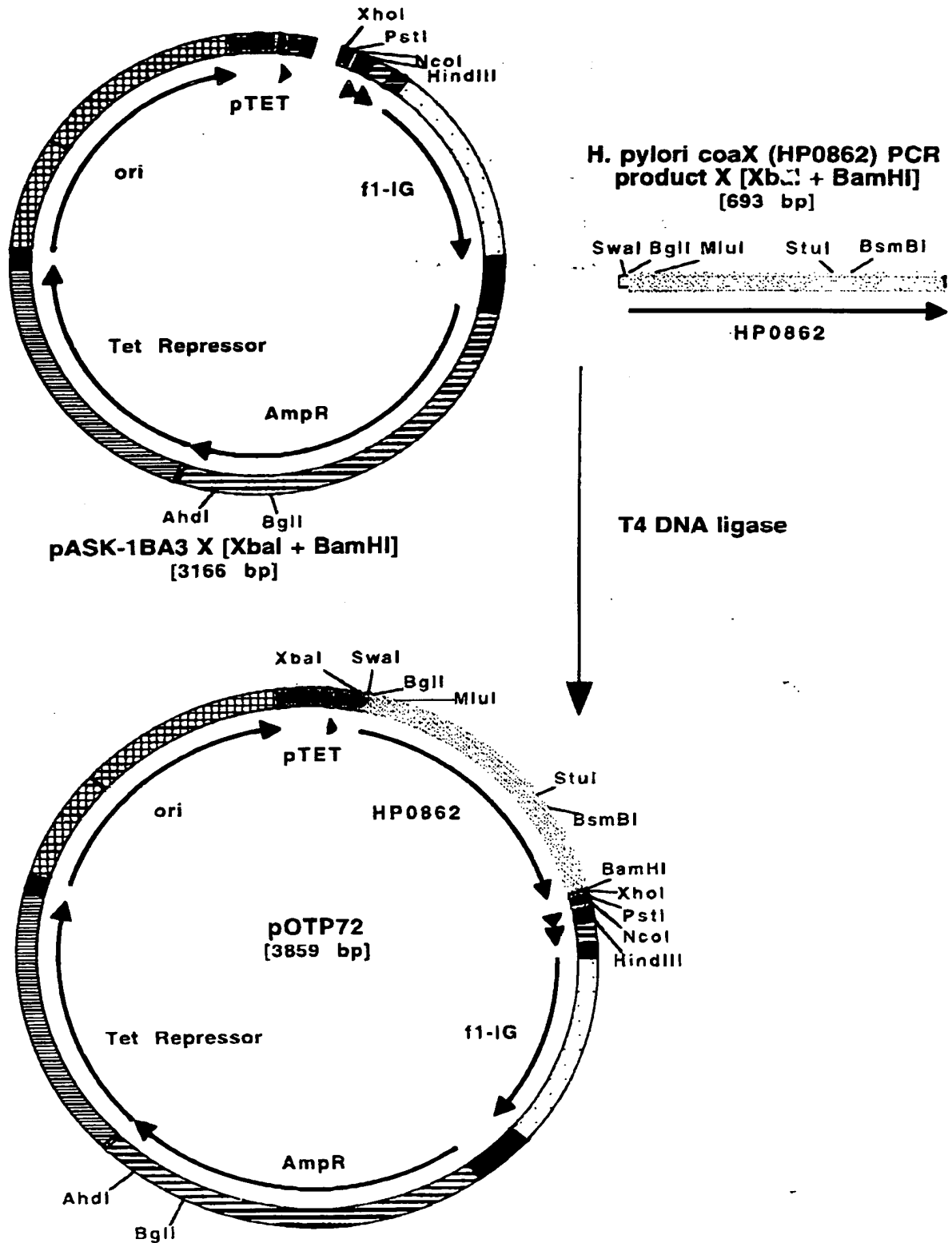
WASRSEVR-----
CFGD-----
ARLVPTSLPPATVSGSSGN
GNSIDFKFVN-----
LYLYHRI-----
HHGDRPIC-----
LKKAGILECK-----
AAQGAPTA-----

Figure 26 Structure of pAN336, a plasmid designed to delete *B. subtilis* *coaX* from the chromosome and replace it with a kanamycin resistance gene.



Construction of pOTP72

Fig 8



Construction of pOTP73

Fig 9

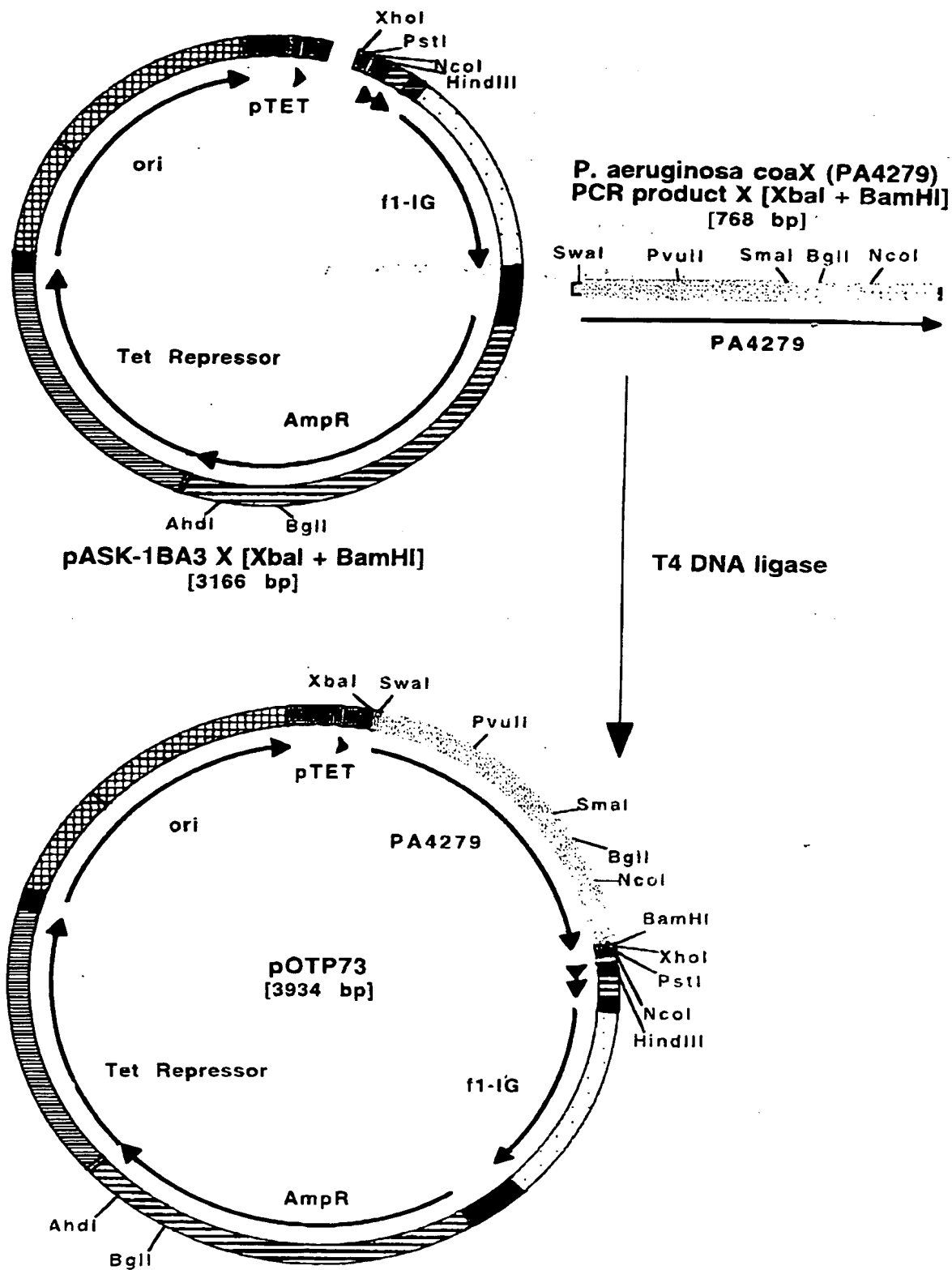


Fig 10

Construction of pOTP71

